Progress report 2022

Next-Generation Precision Medicine Development Laboratory

Project Associate Professor: Hidenori Kage
Project Lecturer: Akiko Kunita

■ Overview

Cancer genomic medicine is the process of undergoing a comprehensive genomic profiling (CGP) test to find gene mutations which can lead to targeted therapy. We have been developing Todai OncoPanel (TOP) at The University of Tokyo Hospital since 2016. Unlike other approved CGP tests which only has a DNA panel, TOP has a dual DNA and RNA panel. Furthermore, many polymorphism probes enable sophisticated gene copy number analysis. Department of Next-Generation Precision Medicine Development Laboratory opened in April 2021 as a social cooperation program, collaborating with Konica Minolta, Inc.

■ Our research

At the Department of Next-Generation Precision Medicine Development Laboratory, we focus on (1) optimization of pathological specimens for CGP tests and (2) further development of TOP.

(1) Best tissue processing practices for CGP tests with RNA panel

Background: Formalin-fixed, paraffin-embedded (FFPE) samples are valuable resources routinely used for pathology and clinical CGP tests. However, they also represent a multistage process that is not standardized, especially for RNA sequencing. Because pre-analytical sample processing ultimately affects the sequencing accuracy, its optimization is essential. Therefore, we evaluated the effects of fixative concentrations, fixation time, and tissue size on DNA/RNA quality.

Methods: Pig livers were removed within 30 min of sacrifice. Equally sized tissues (2 mm/ 12 mm/ 50 mm thickness) were fixed in neutral-buffered formalin (10% or 20% NBF) for one (24 h), three (72 h), and seven days (168 h). FFPE blocks were prepared, and DNA and RNA were extracted from each 10 μm section using spin columns. The DNA/RNA quality was evaluated using fluorescence-based quantification and fragment size distribution (DIN for DNA/DV200 for RNA).

Results: DNA integrity was best for samples fixed in 10% NBF for one day. The RNA integrity remained unchanged irrespective of the NBF concentration. For RNA, the best fixation duration was one day. Increased degradation was observed in both DNA and RNA extracted from samples fixed for three and seven days versus those fixed for one day. Regarding tissue size in fixation, DNA yield per tissue area and the integrity were best extracted from small (2 mm thickness) tissues and had a great influence over the integrity. Conversely, RNA yield per tissue area and the integrity were best when extracted from large (50 mm thickness) tissues and had the minimal impact on the integrity.

Discussion: Effects of the fixation methods varied between DNA and RNA. For the best tissue processing practices, fixation in 10% NBF for one day was suitable for DNA and RNA. Large tissues should be sliced into thin sections for effective fixation.

(2) Clinical utility of Todai OncoPanel in the setting of approved comprehensive cancer genomic profiling tests in Japan

Background: Comprehensive cancer genome profiling (CGP) has been nationally reimbursed in Japan since June 2019. Less than 10% of the patients have been reported to undergo recommended treatment. Todai OncoPanel (TOP) is a dual DNA-RNA panel as well as a paired tumor-normal matched test.

Methods: Two hundred patients underwent Todai OncoPanel as part of Advanced Medical Care B with approval by the Ministry of Health, Labour and Welfare between September 2018 and December 2019. Tests were performed in patients with cancers without standard treatment or when patients had already undergone standard treatment.

Results: Data from DNA and RNA panels were analyzed in 198 and 191 patients, respectively. The percentage of patients who were given therapeutic or diagnostic recommendations was 61% (120/198). One hundred and four samples (53%) harbored gene alterations that were detected with the DNA panel and had potential treatment implications, and 14 samples (7%) had a high tumor mutational burden. Twenty-two samples (11.1%) harbored 30 fusion transcripts or MET exon 14 skipping that were detected by the RNA panel. Of those thirty transcripts, six had treatment implications and four had diagnostic implications. Thirteen patients (7%) were found to have pathogenic or likely pathogenic germline variants and genetic counseling was recommended. Overall, 12 patients (6%) received recommended treatment.

Discussion: In summary, patients benefited from both TOP DNA and RNA panels while following the same indication as the approved CGP tests.

■ Future directions

(1) Optimization of pathological specimens for CGP tests

We aim to standardize the storage conditions for unstained FFPE tissue slides by evaluating the effects of storage time and temperature on DNA/RNA quality.

(2) Further development of TOP

We have started a prospective study using TOP as a run-through test. We plan to continue the study focusing on homologous recombination deficiency and compare the performance of TOP with approved tests.

■ Research activities for fiscal year 2022

Publications:

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